

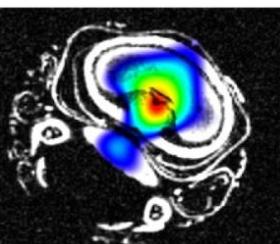
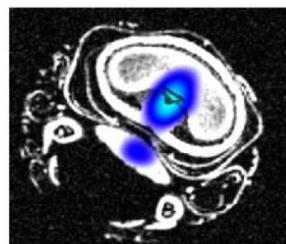
High-throughput screening of cereal collections for abiotic stress tolerance



Kerstin Neumann, IPK
2019 International Spring School "Computational Biology
Starter"

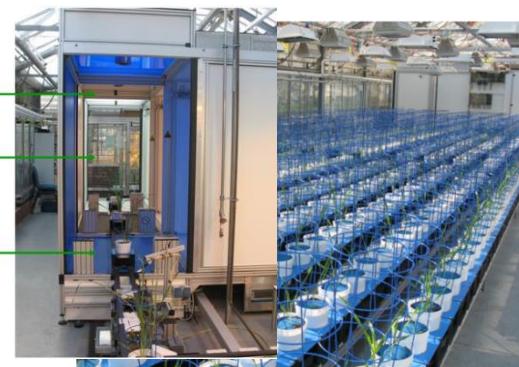
Research Infrastructure - Phenotyping

NMR:
grains



Microscopy:
Leaf epidermal cells

Greenhouse:
Images
RGB, Fluor, NIR, IR



Plant cultivation hall – world-wide unique, field-like controlled conditions



Field: AgRover equipped with hyperspectral camera



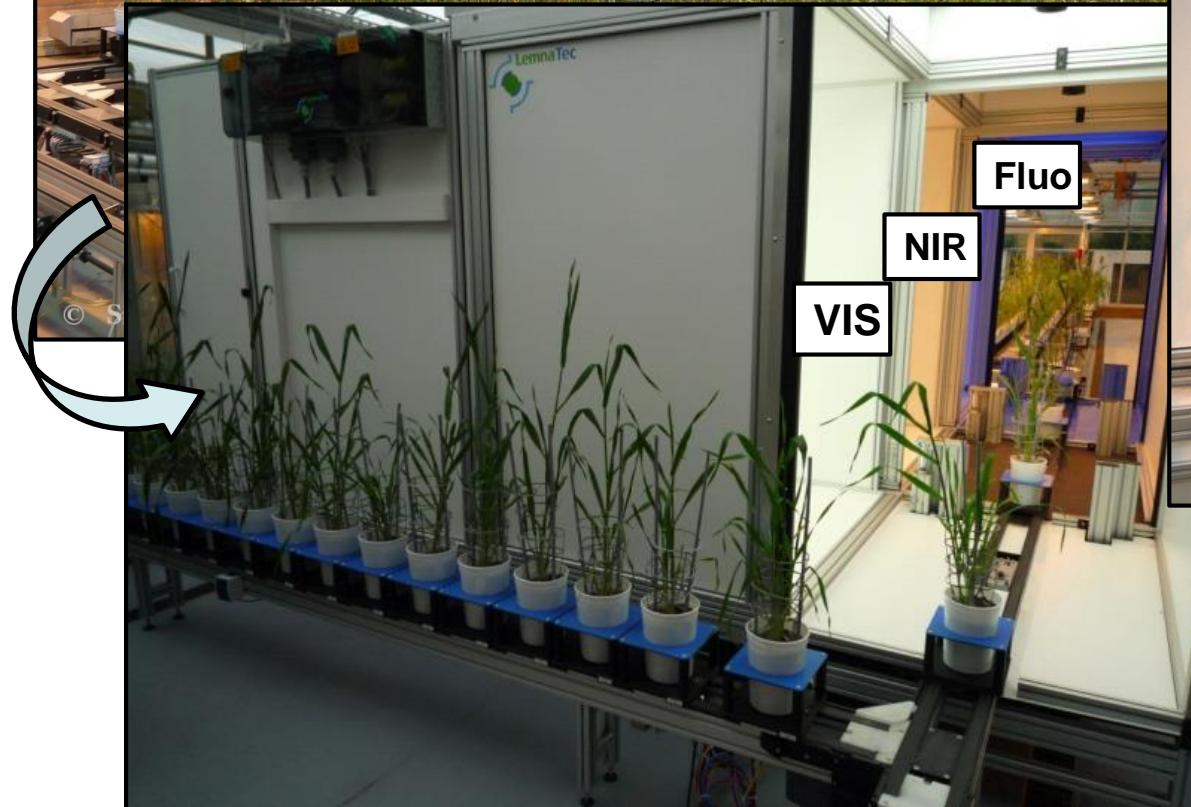
➤ IPK is part of many phenotyping networks: DPPN, EPPN, IPPN and EMPHASIS



HTP system employed mainly for cereals

520 pots, 1 plant / pot

Automated randomization



Automated watering

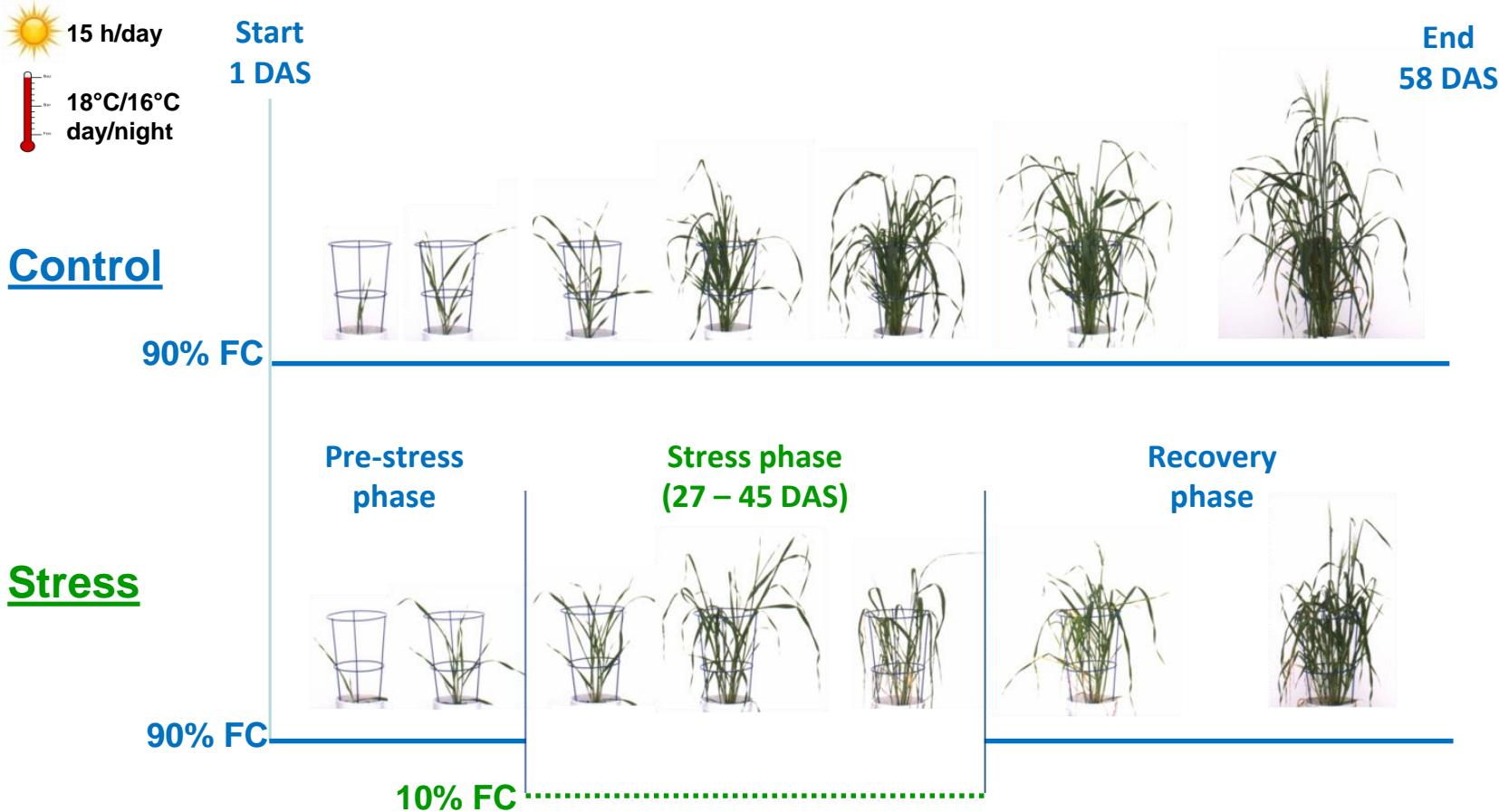


Development of cereal phenotyping

- Setup mimicing a spring drought event established on a barley core set of 18 accessions (Chen et al. 2014, Neumann et al. 2015)
- Genetic architecture in a spring barley collection of 100 diverse two-rowed IPK accessions in optimal (Neumann et al. 2017) and drought conditions (Dhanagond et al. to be submitted), 9k iSelect SNPs
- Setup transferred and adapted to a winter wheat collection of 220 cultivars, 15k iSelect SNP array (now 135k array), Phase 2 heat
- Collection of 130 spring wheat genotypes, spring and late drought setup (exome capture SNPs)

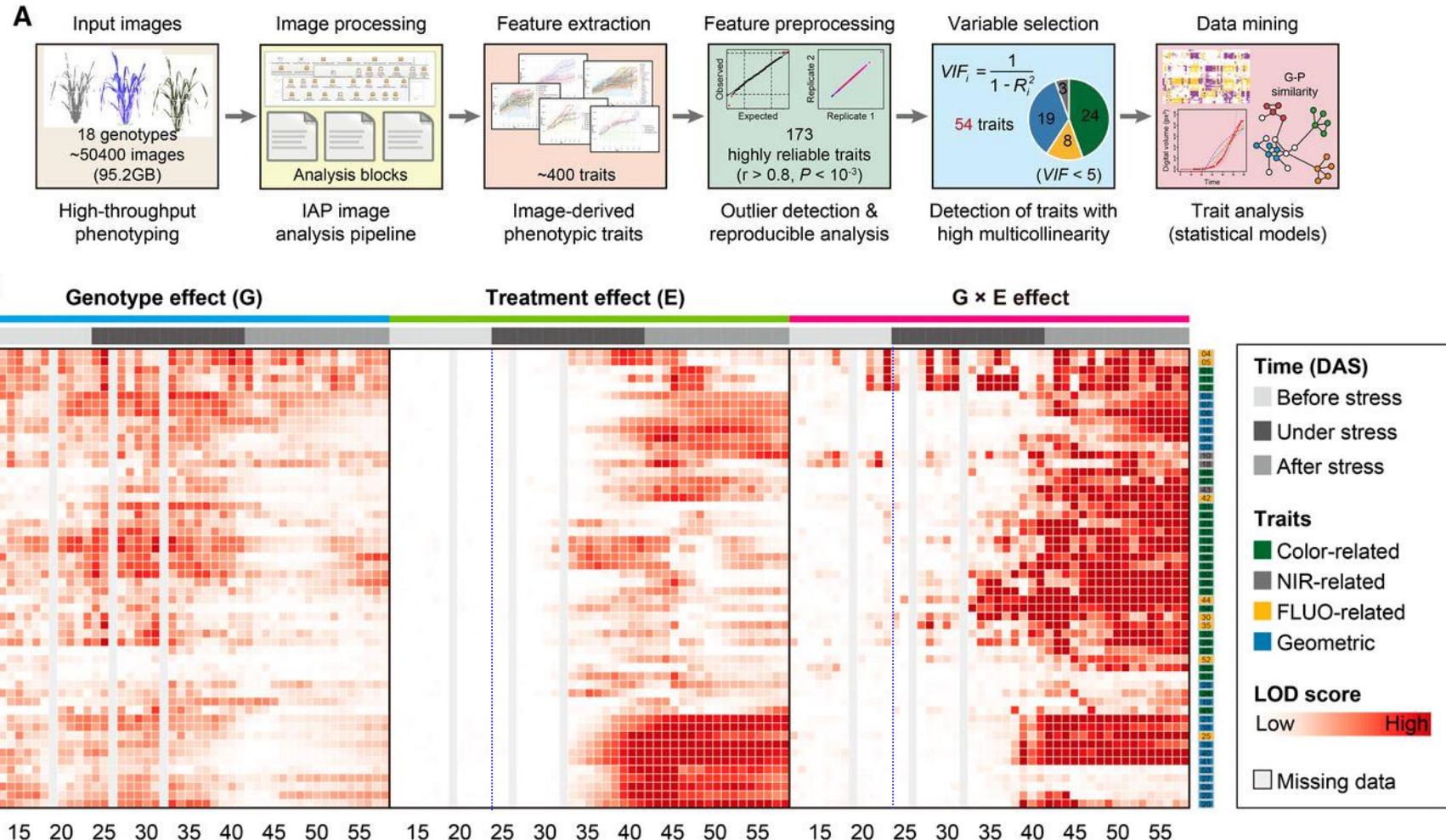


Spring barley setup for drought phenotyping



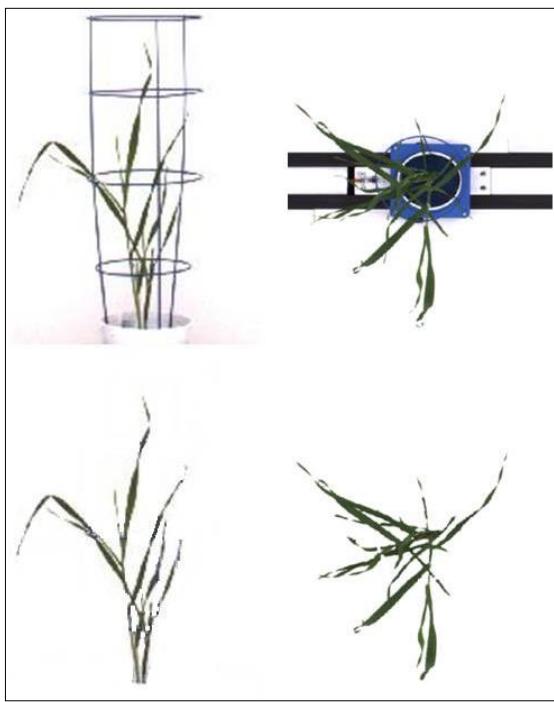
- Daily imaging and watering
- 4 Image configuration: 1 top view, 3 side views
- Randomization by system modi – randomized design

Data amount in phenotyping is challenging



Chen et al. 2014 *The Plant Cell*, Vol. 26: 4636–4655

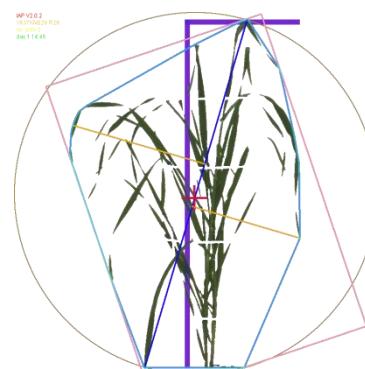
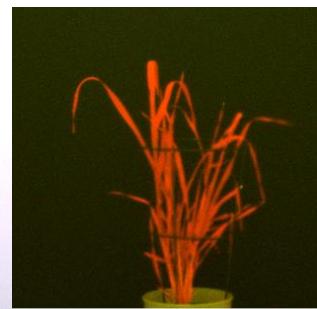
From raw images to phenotypic traits



VIS

Fluo

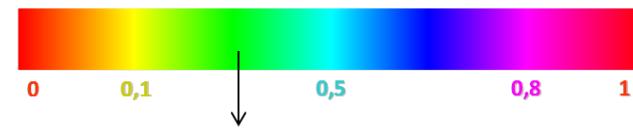
NIR



- Digital biomass
- Plant height and – width
- Compactness
- Parameters of convex hull
- Colour classification

→ Flowering: not yet automatically
→ Number of tillers manually counted

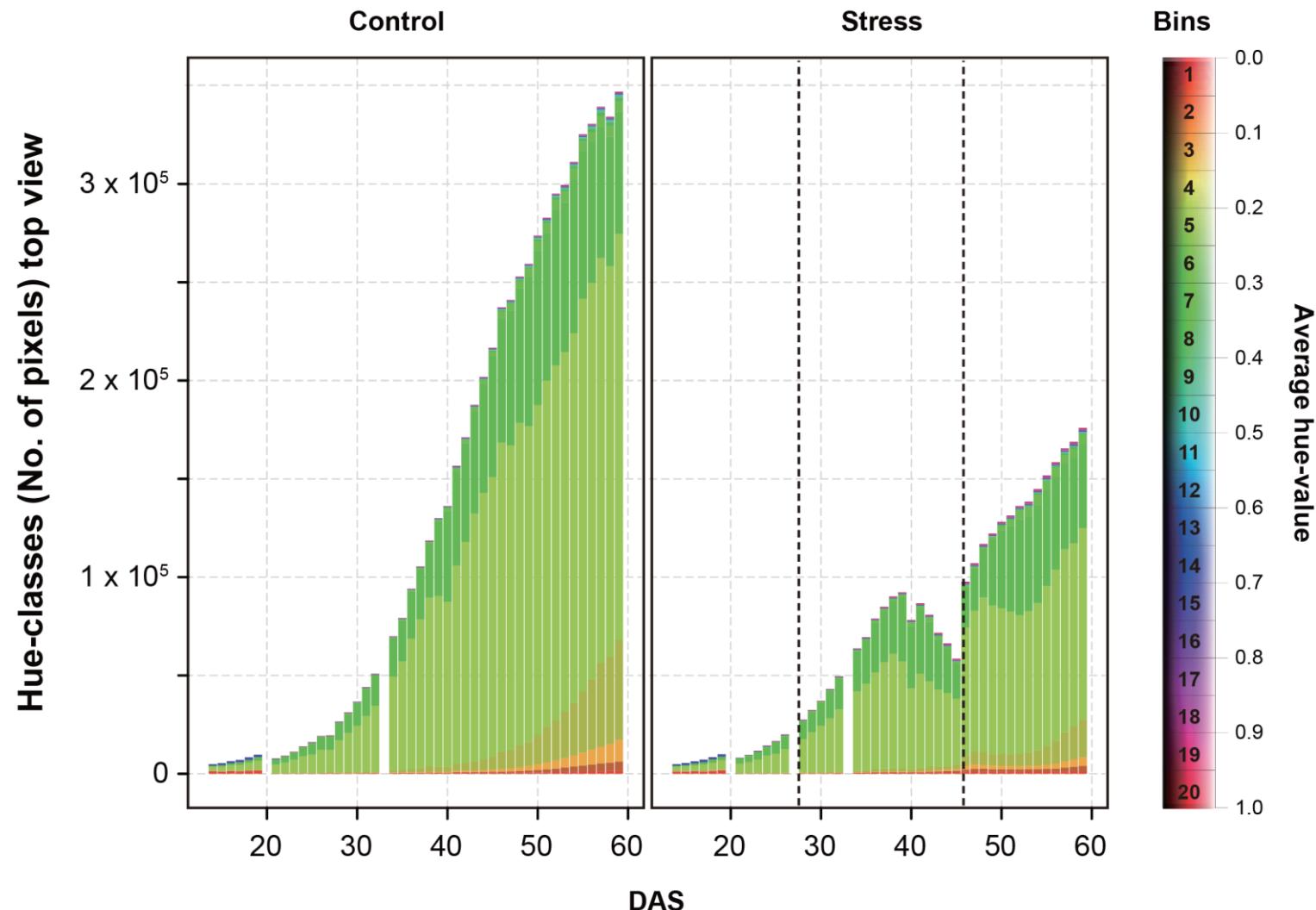
Average hue-value



Green plant: hue ~ 0.23

IAP: Integrative Analysis Platform
(Klukas et al. 2014, *Plant Phys*)

Examples of trait observation over time – color (RGB)



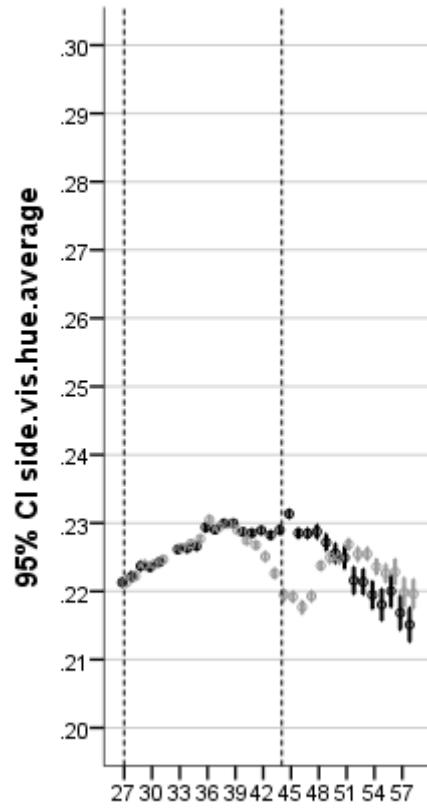
Neumann et al. 2015 *Plant Cell Env*, Vol. 38: 1980-1996

Examples of trait observation over time – color (RGB)

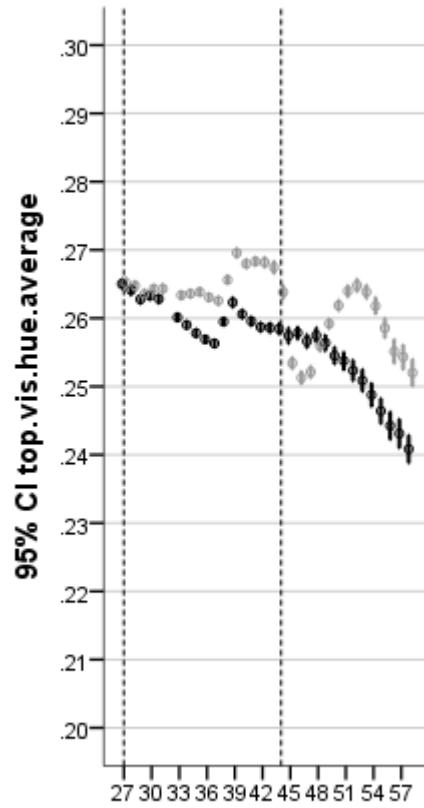
Control
Stress

Side vs top view

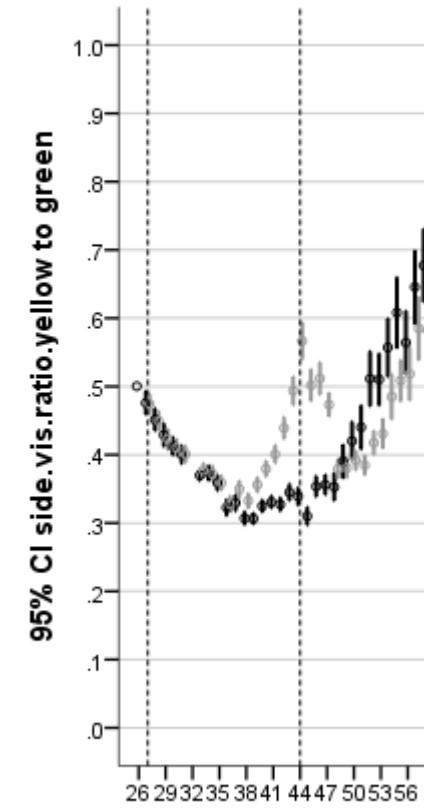
HUE-side



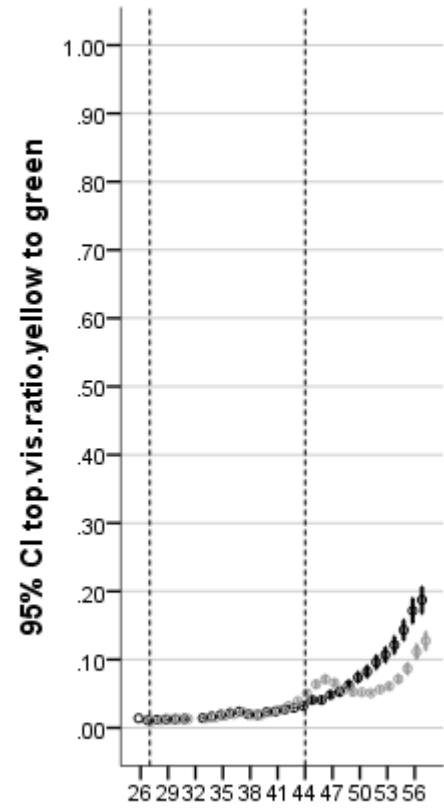
HUE-top



YtG-side



YtG-top



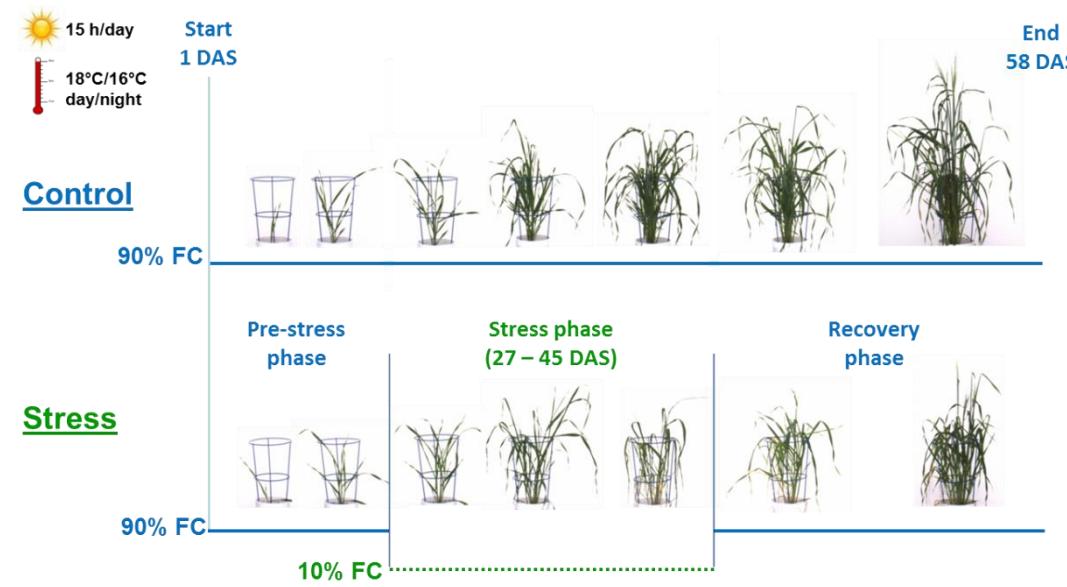
Average color value

Yellow to green ratio

Neumann et al. 2015

One trait alone reveals many information

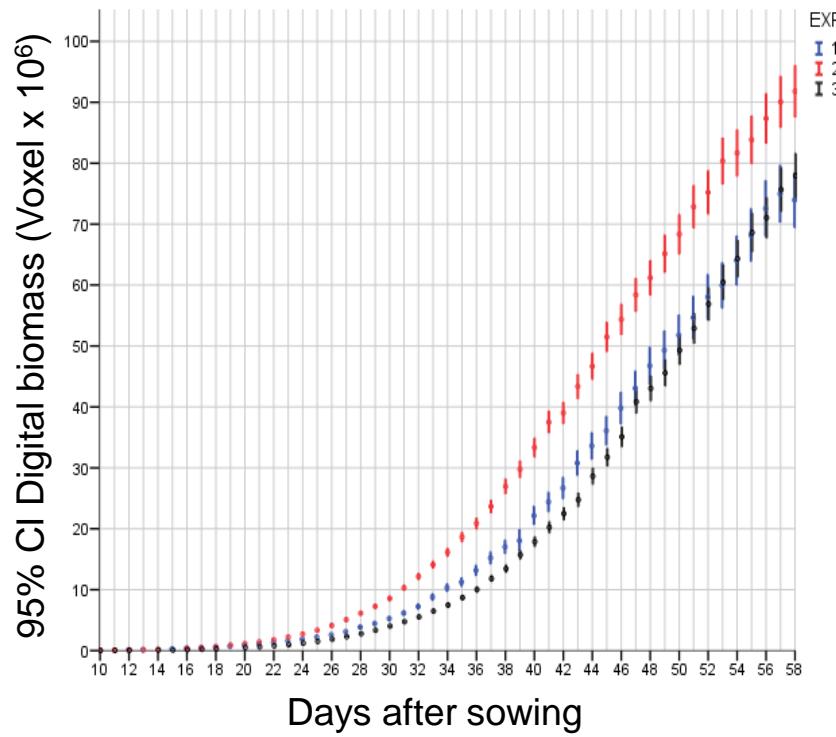
Observation of plant growth – biomass over time



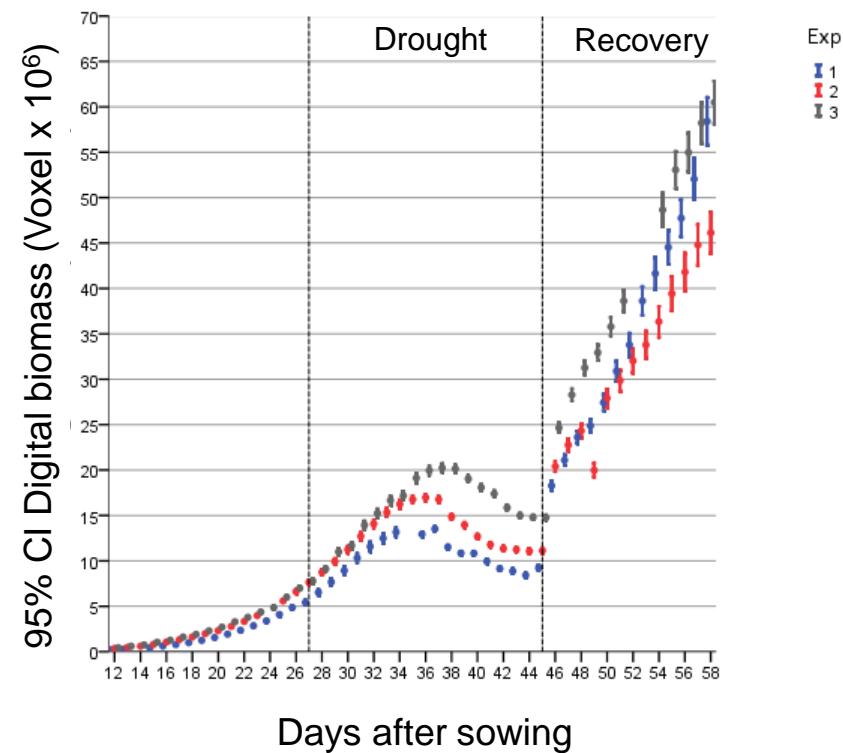
- 100 genotypes
- 3 experiments for each treatments
- 5 plants per genotype
- Analysing biomass day by day as a trait: outlier within, across experiments, calculation of BLUEs, variance components, heritability, GWAS with FDR
- Biomass curves of single plants for growth modelling to extract new phenotypic traits

Growth in barley – pixel volume as proxy for biomass

Well-watered treatment



Spring drought treatment



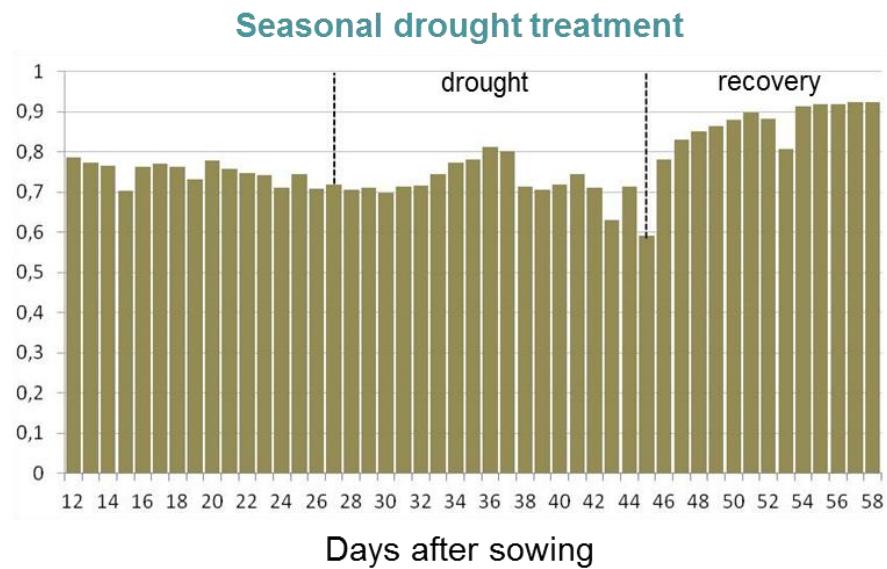
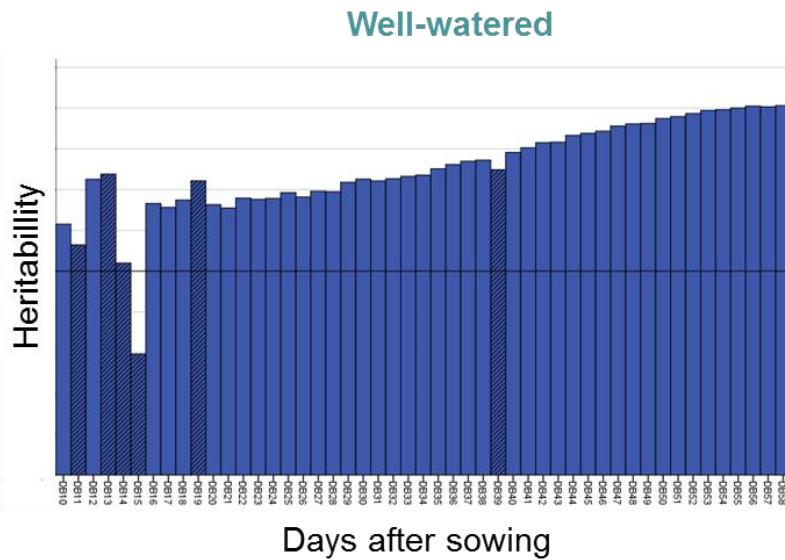
- Based on BLUES values of accessions within the single experiments

$$\text{Digital biomass} = \sqrt{\text{average pixel side area}^2 * \text{top area}}$$

High data quality emphasizes genetic studies

$$\frac{V_G}{V_G + \frac{V_{GE}}{O} + \frac{V_e}{OR}}$$

V_G , V_{GE} , and V_e = variance components of genotype, genotype x experiment and the residual, O = number of experiments (3), R = the number of replicates (5)



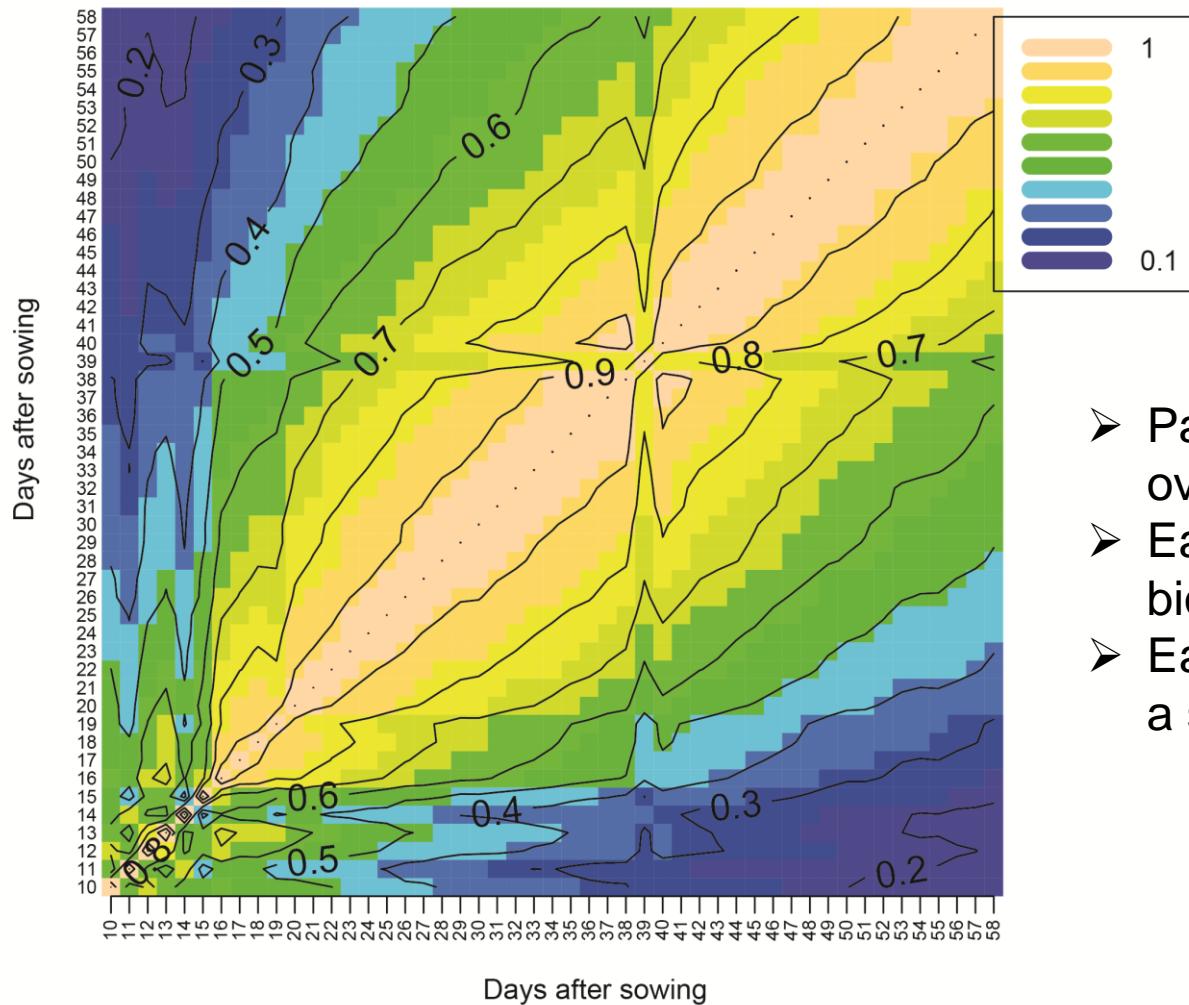
Hatched bars = DAS with missing values

Broad sense heritability range for biomass over time: 0.59 – 0.91 → suitable for GWAS



Phenotypic correlations of biomass at different days

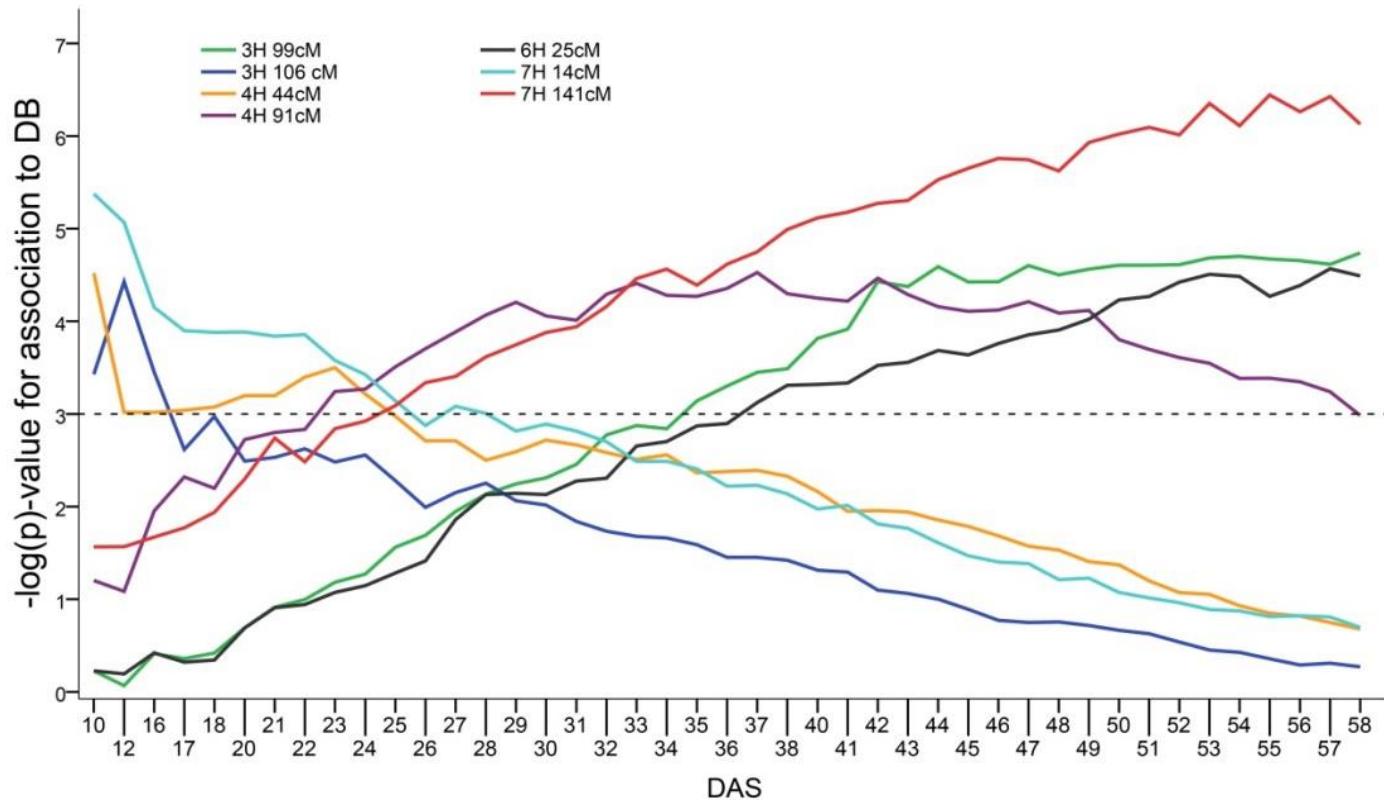
Kendall correlation of digital biomass



- Pattern changes over time
- Early and late biomass not related
- Early biomass shows a stronger dynamic

Neumann et al. 2017 BMC Plant Biol, 17:37

QTL mapping for biomass over time by GWAS in two-rowed spring barley collection

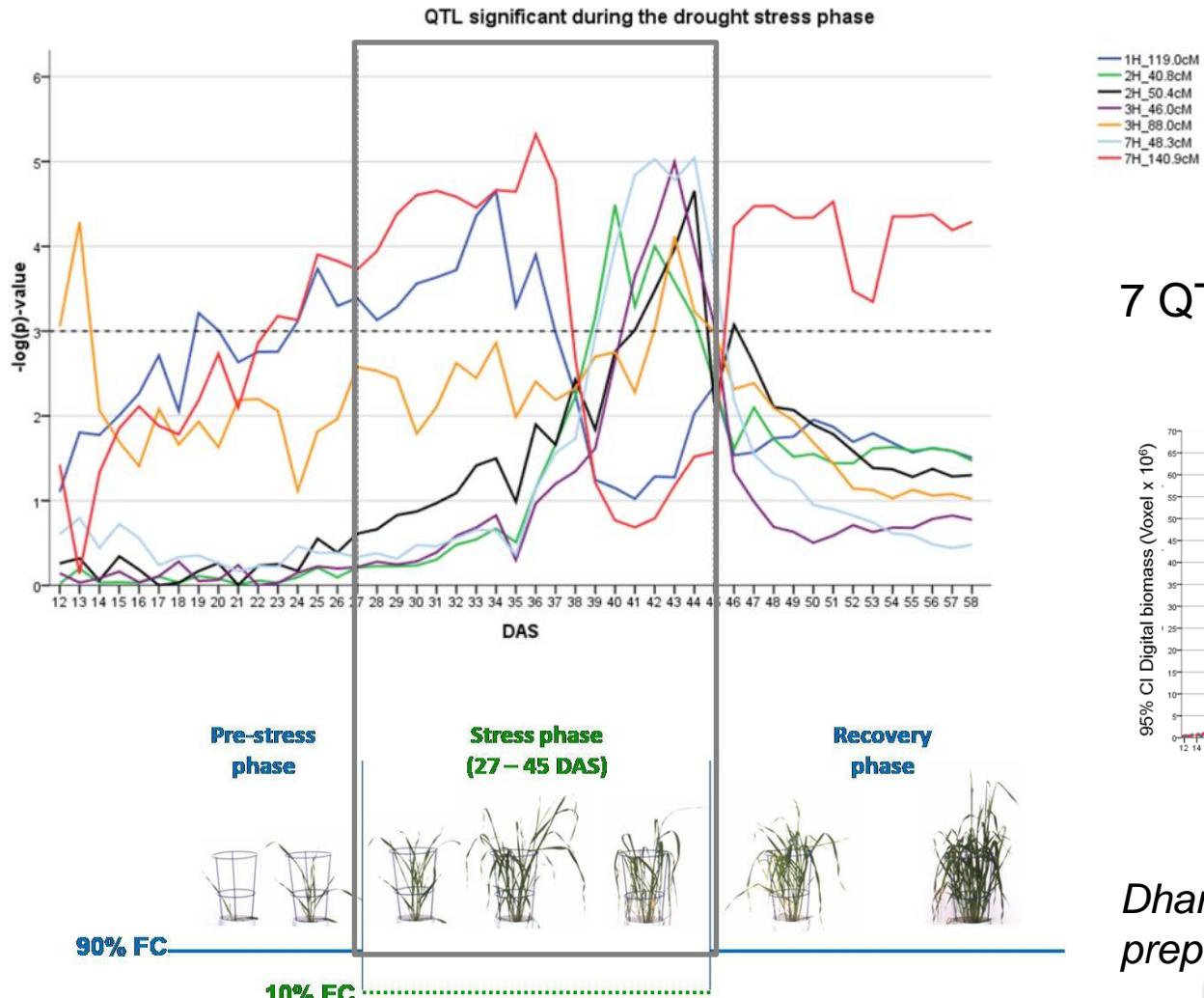


- **7 QTL** significant minimum during one time point, 3 for early, 4 for later development
- Explain collectively **44-52%** of genetic variance

Neumann et al. 2017

Genetic architecture of vegetative biomass in spring barley during stress conditions

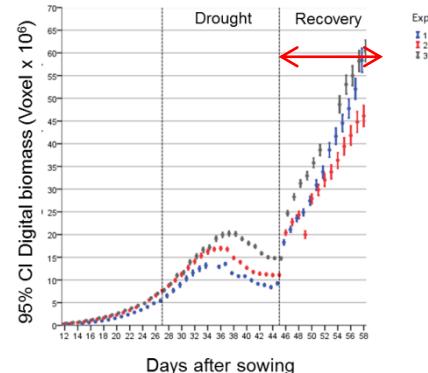
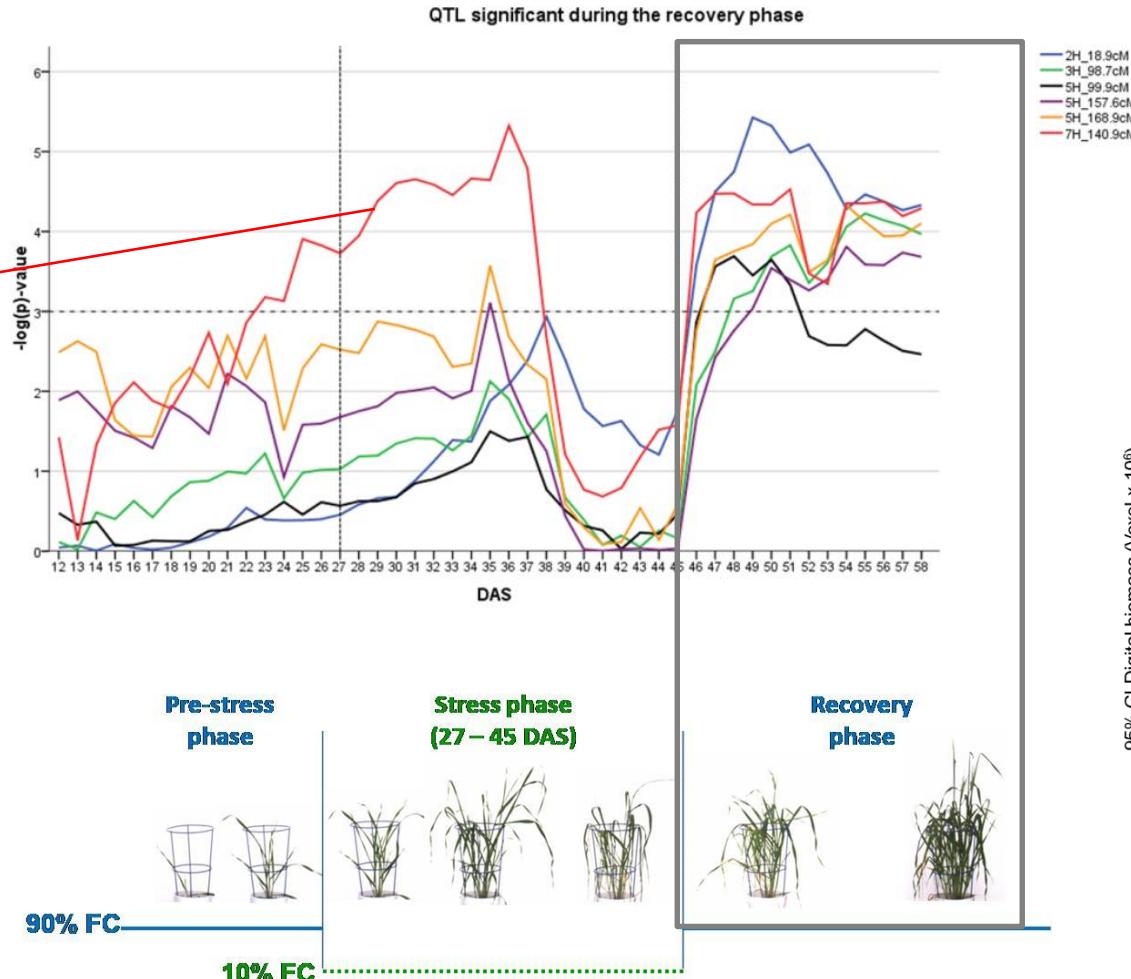
QTL during drought stress phase



Genetic architecture of vegetative biomass in spring barley during recovery from drought

QTL during recovery phase

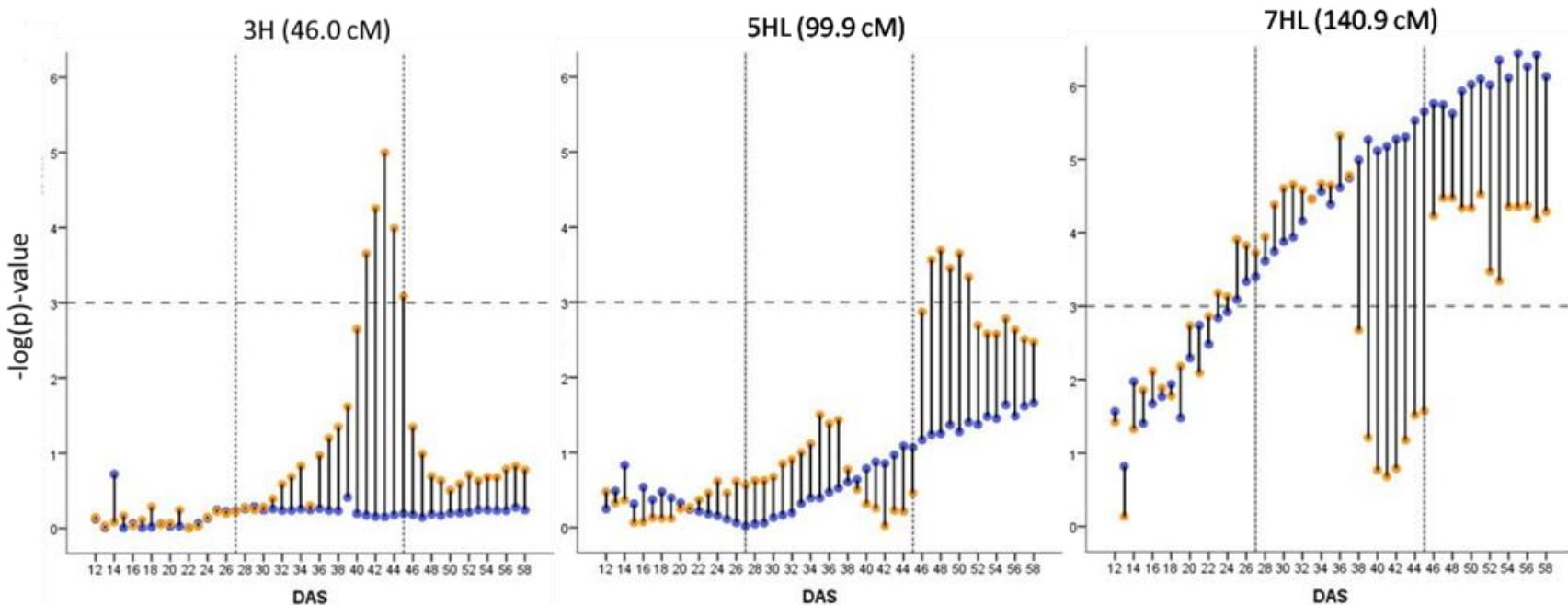
Only 1 QTL
shared by
both phases



Dhanagond et al. in
prep

Classification of identified QTL in respect to their appearance (spring barley example)

Drought adaptive QTL (4) Recovery adaptive QTL (1) Constitutive QTL (8)

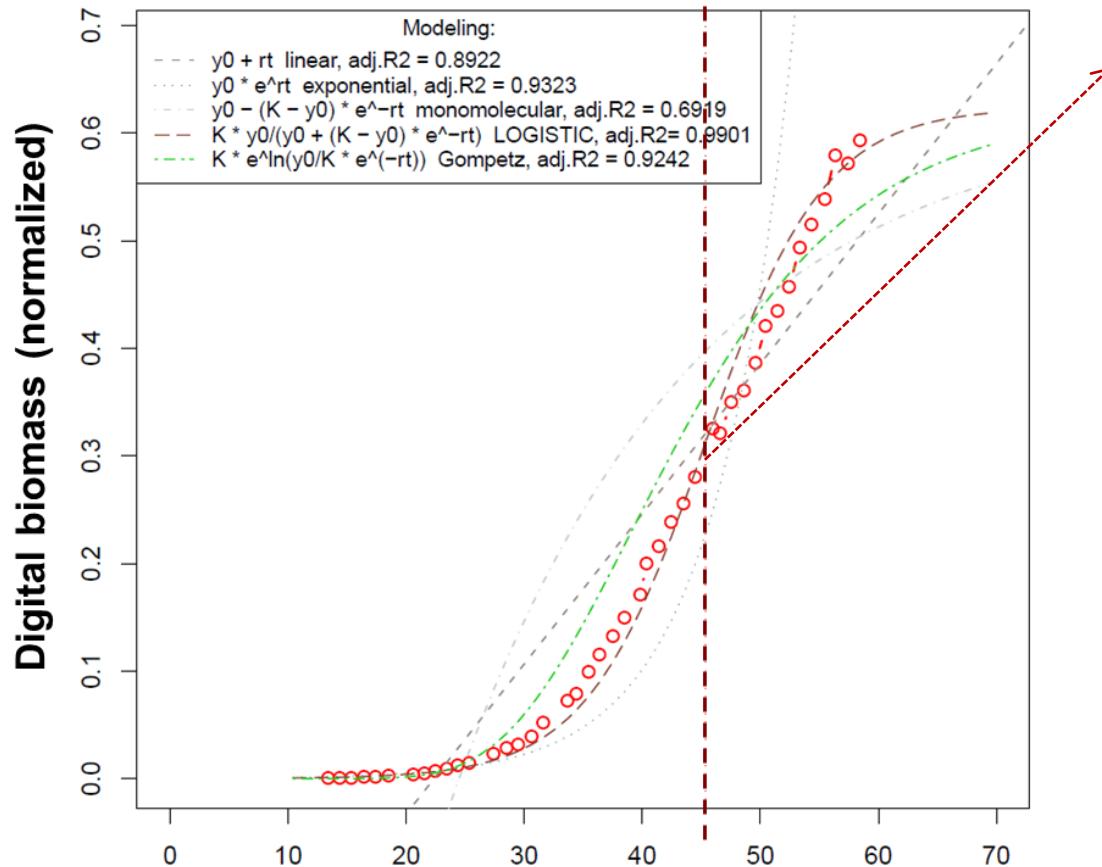


- 4,866 informative SNPs from 9k iSelect array barley panel, GWAS with MLM including kinship, FDR 0.1
- horizontal dashed line indicates $-\log(p) = 3$

Control
Stress

Biomass growth modelling - control

Well-watered treatment



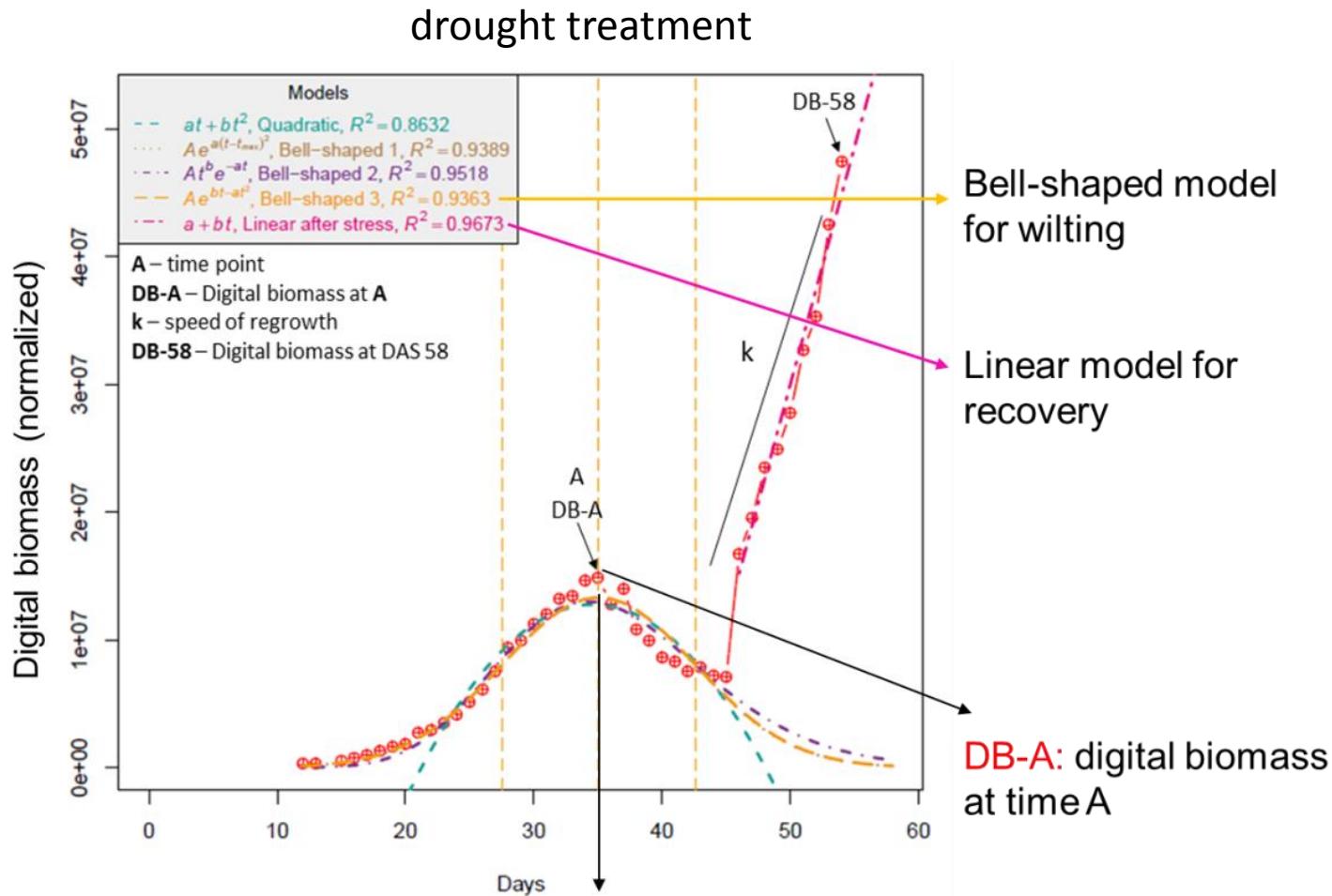
Inflexion point:

point where growth is maximal, before ever increasing, afterwards slowing down

Range of ~ 9 days in barley collection

Revealed 2 independent QTL in vicinity of flowering time genes

Biomass growth modelling - stress



Time point A: maximum biomass in stress phase, stop of growth, „wilting time“

→ Range of ~6 days, revealed 4 QTL, 2 co-located with biomass, 2 independent

Summary

- High data quality during HTP
- Genetic architecture is time-dependend
- Different QTL in control and stress treatment
- Data amount is challenging
- ➔ Need of developing standardized and as far as possible automatic analysis processes
- ➔ Need of developing interactive QTL browser
- ➔ When is a QTL really significant? New ways of defining significance thresholds in such data sets?
- ❖ Collaboration with image analysis group on ear detection, pollen (flowering), leaf counting – ground truth data sets generated in winter wheat

Acknowledgments



Genome diversity group in IPK:

- Andreas Graner, Benjamin Kilian
- Michele Grieco
- Sidram Dhanagond, Celestine Wabila
- Technicians of GED: Kerstin Wolf, Ute Krajewski, Katrin Trinka, Heike Harms

Gardener team of K. Gramel-Koch/ K. Tiemann

Image analysis group (IPK)

- Former group members Christian Klukas, Dijun Chen, Jean-Michel Pape, Ingo Mücke
- Recent group members: Evgeny Gladilin, Michael Henke

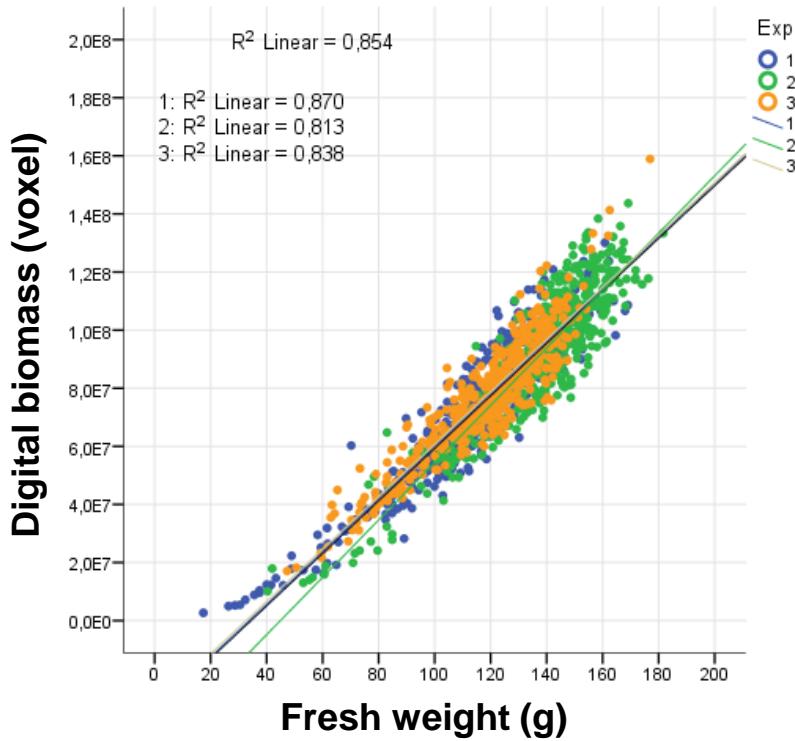
Quantitative Genetics group (IPK)

- Jochen Reif, Yusheng Zhao, Jianting Chu, Guozheng Liu

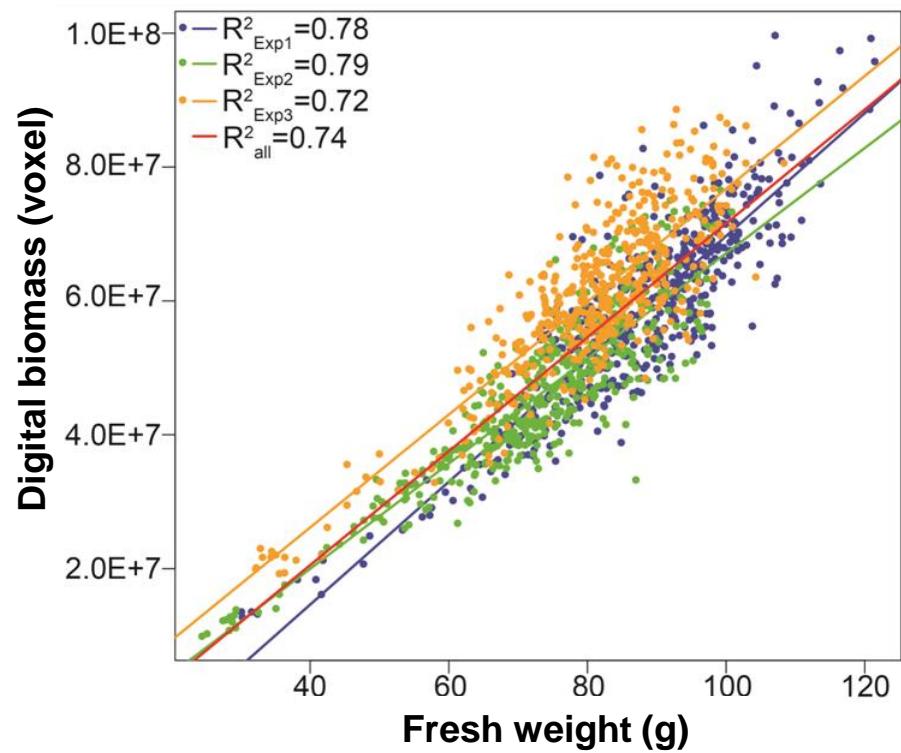


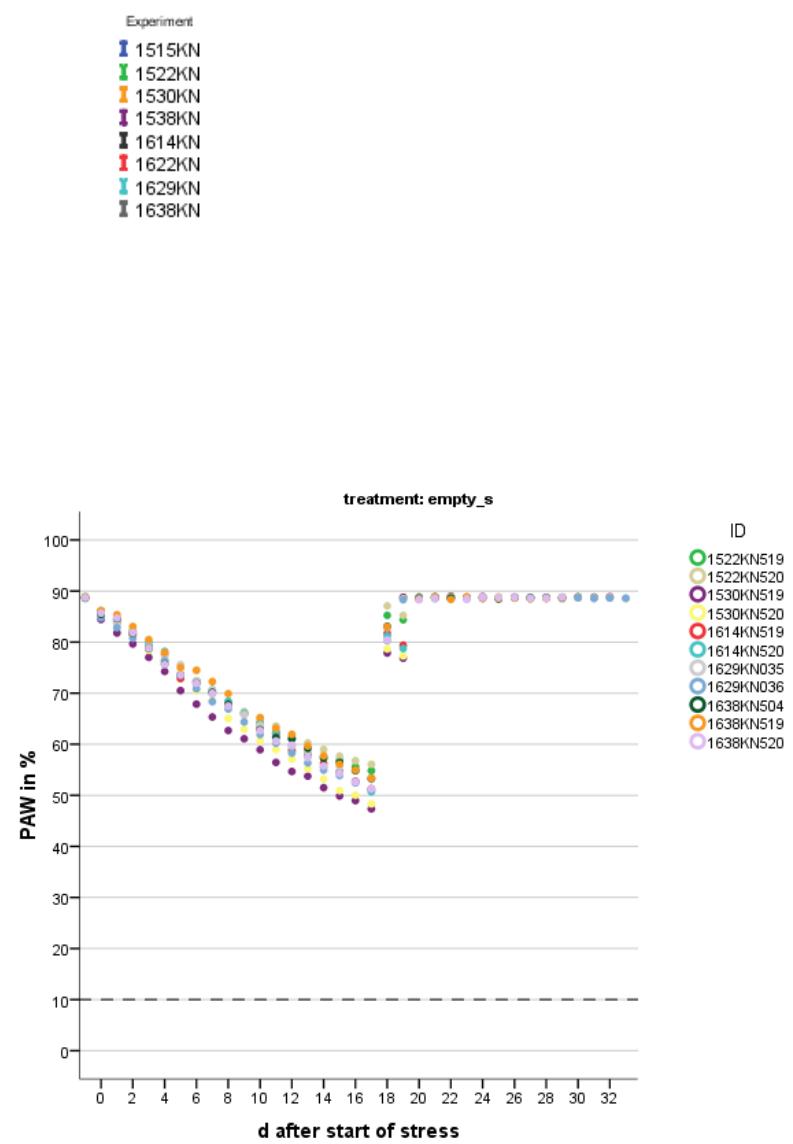
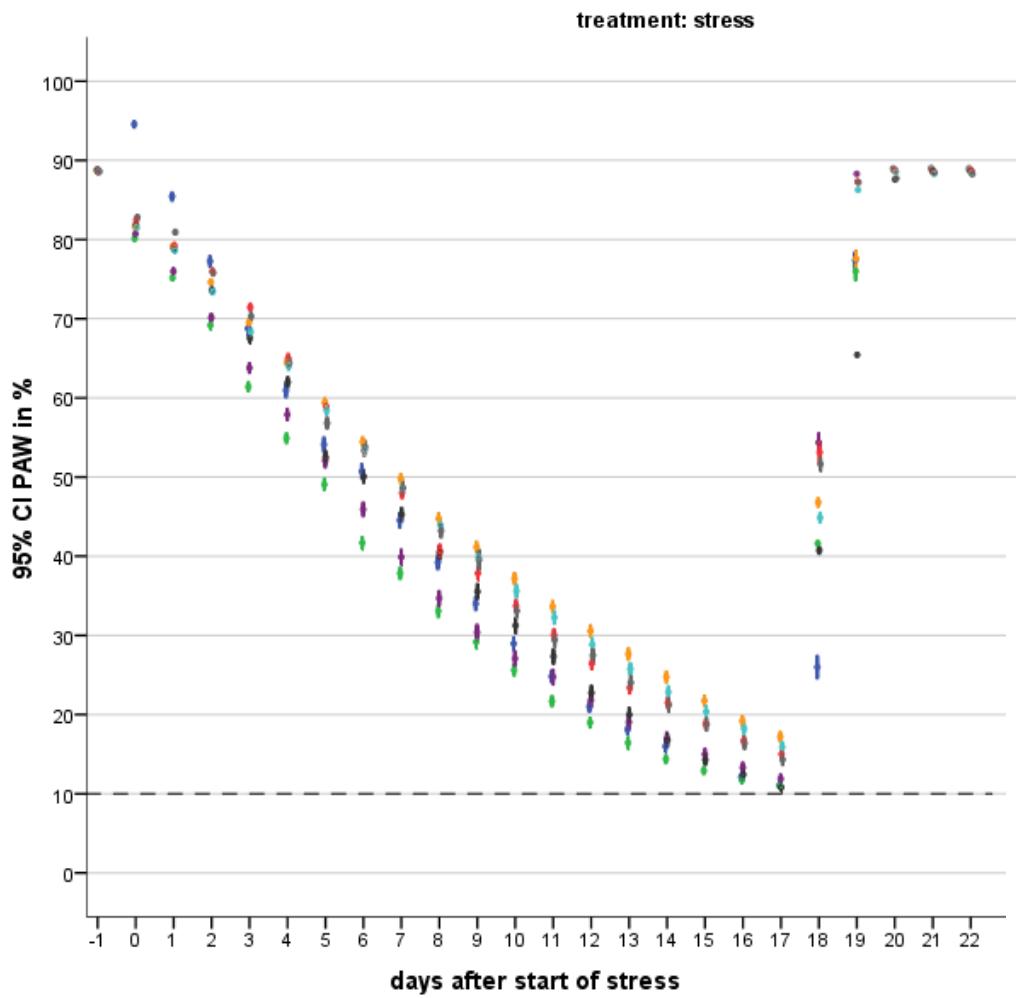
Data quality check – Correlation fresh weight and digital biomass at DAS 58

Control treatment (1500 plants)



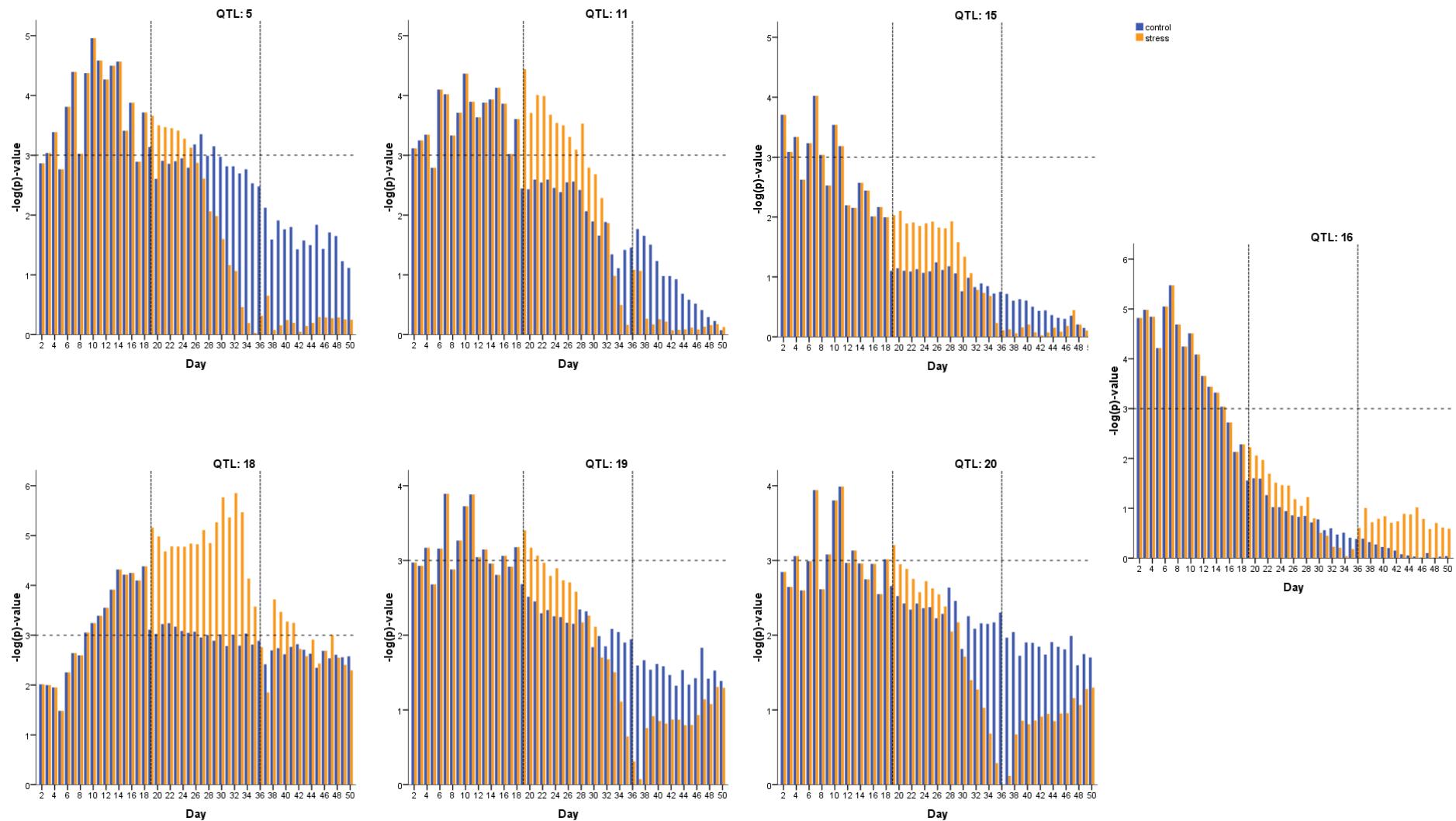
Stress treatment (1500 plants)





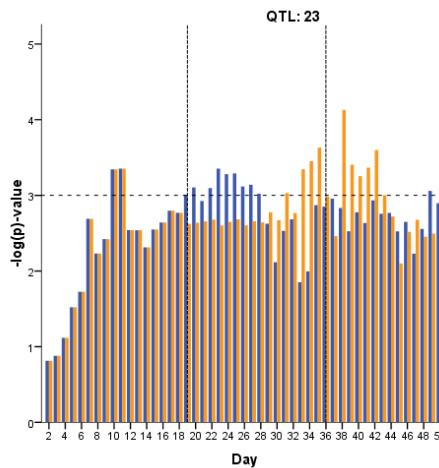
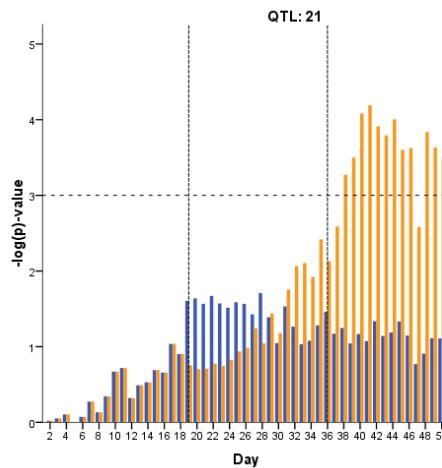
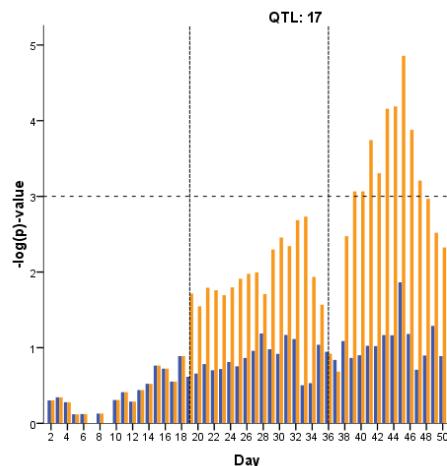
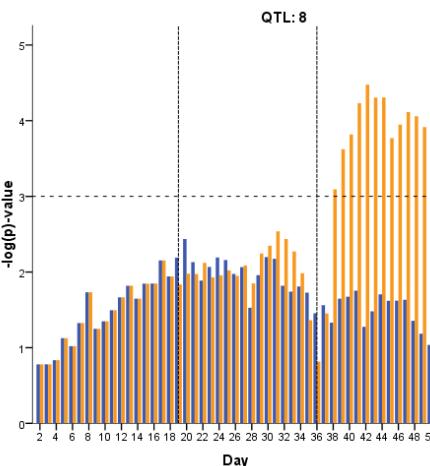
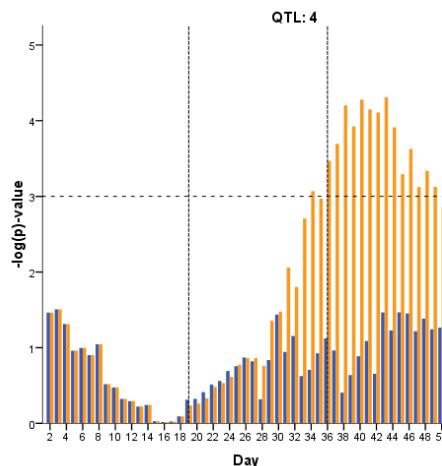


Early biomass QTL in winter wheat before stress



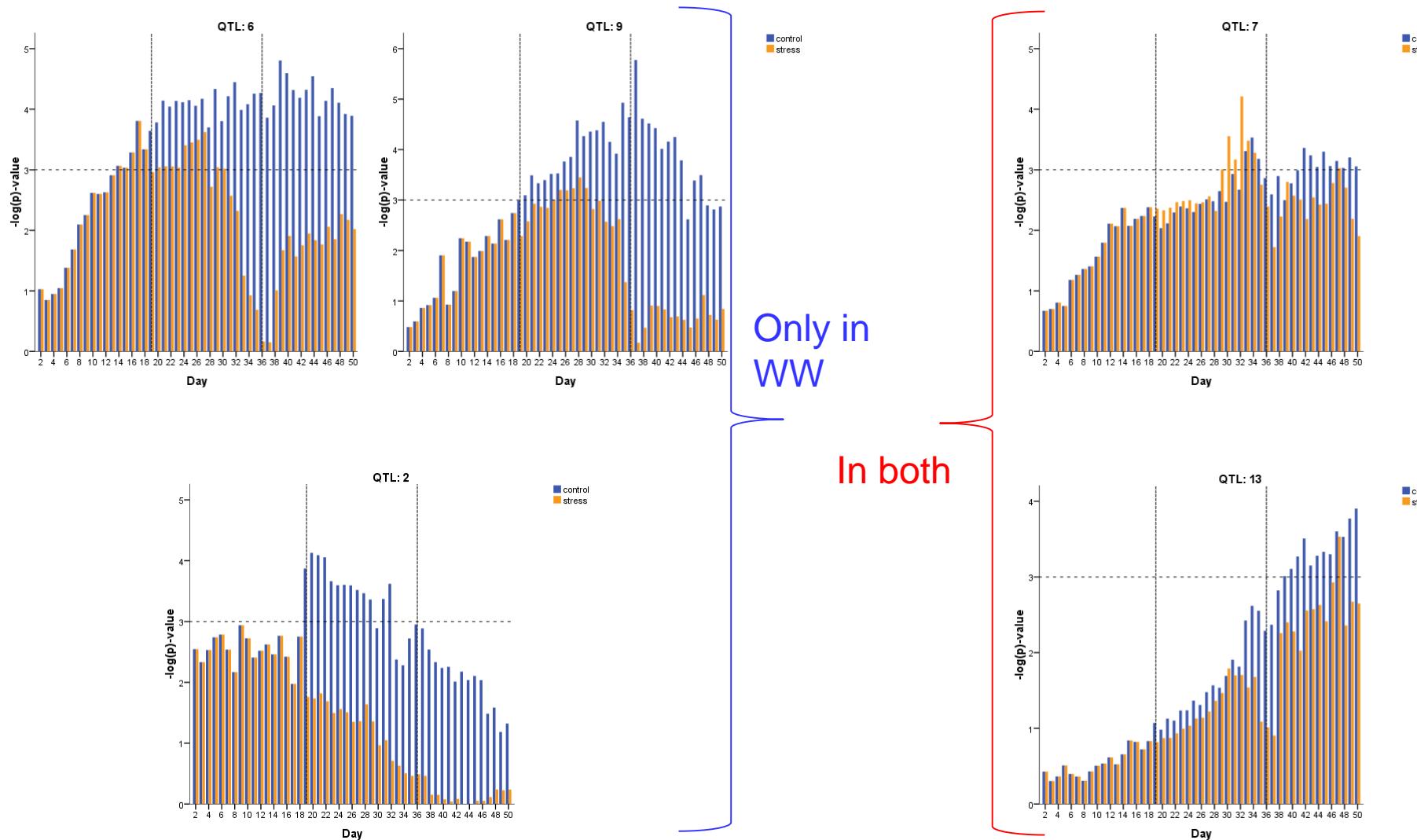


Recovery-related biomass QTL in winter wheat



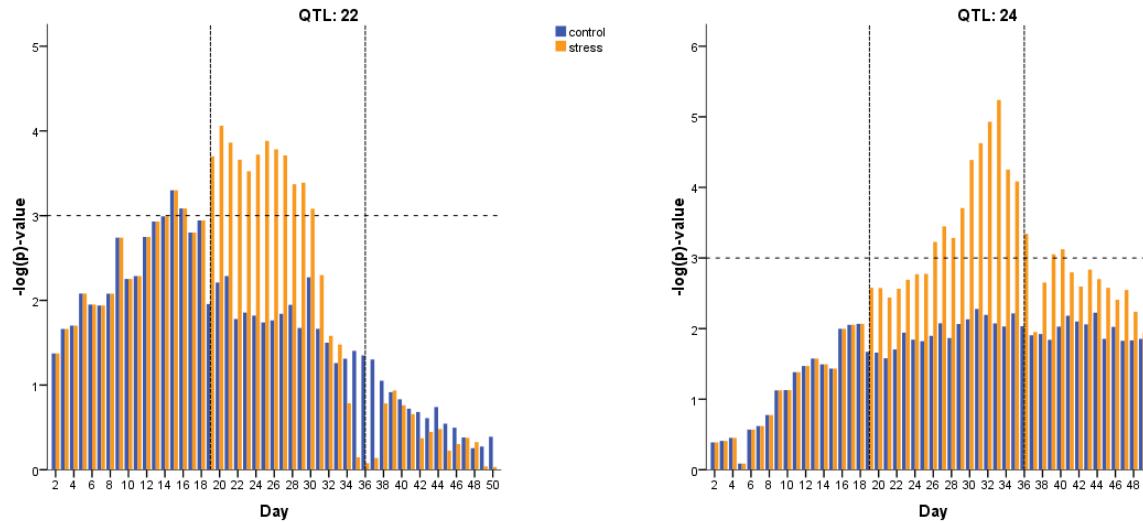
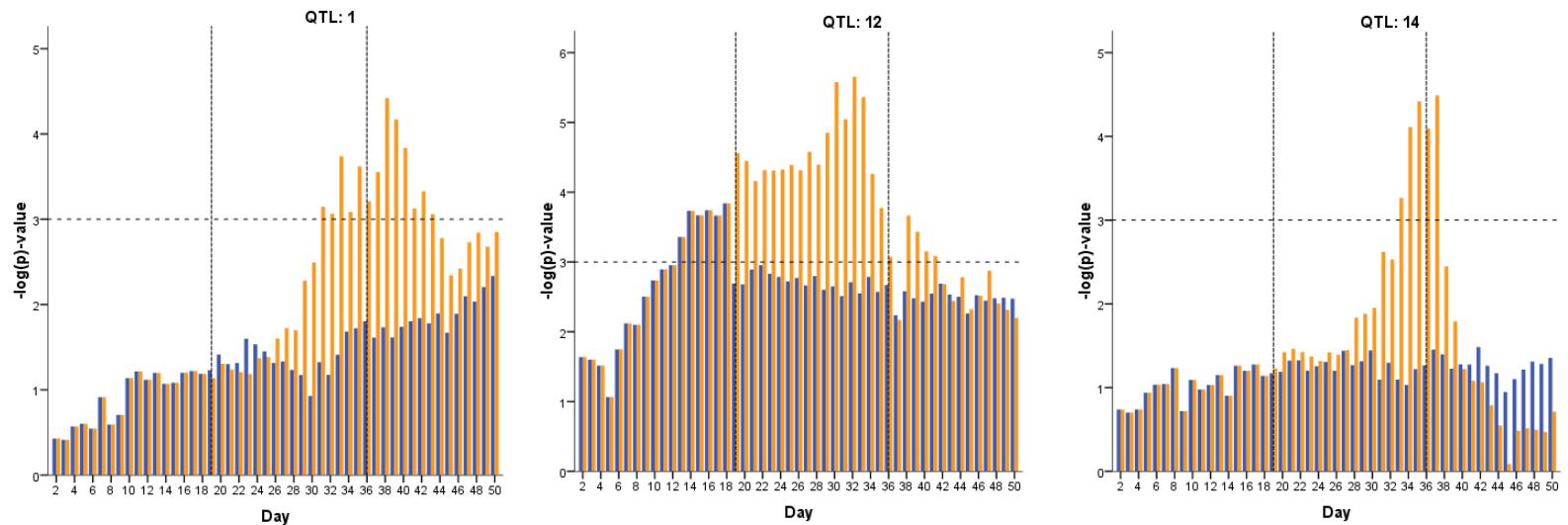


Drought independent biomass QTL in winter wheat



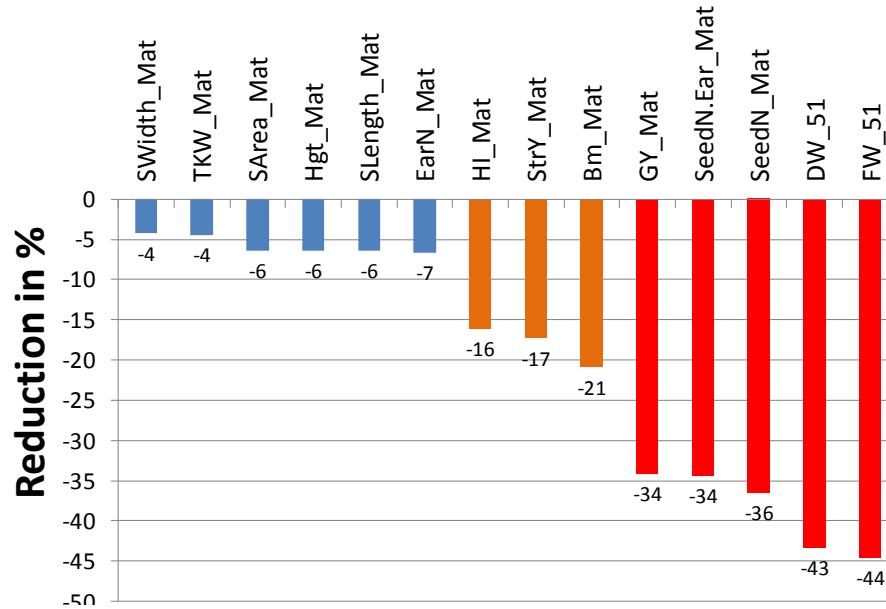
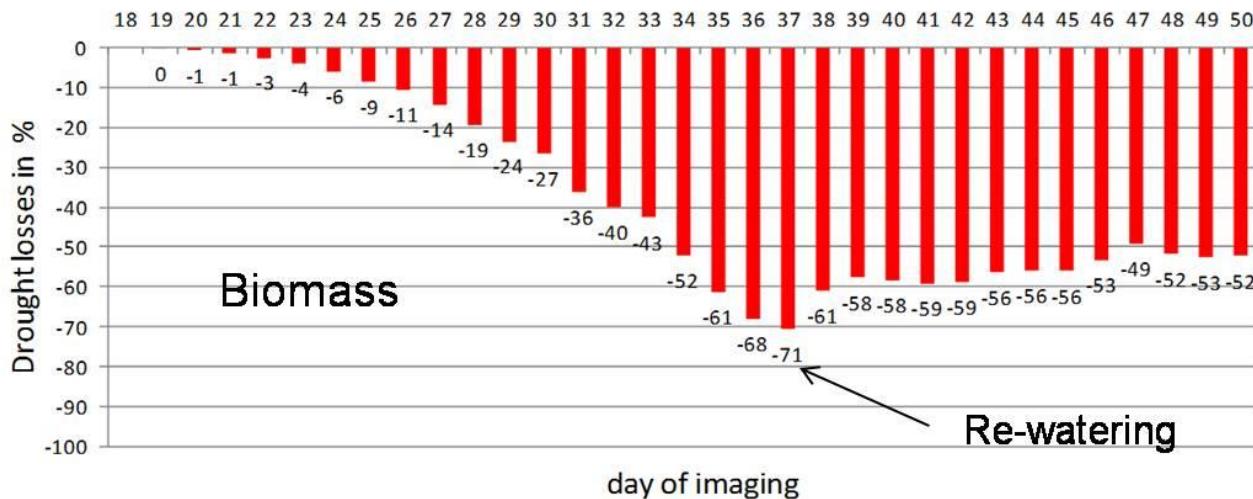


Drought-related biomass QTL in winter wheat





Trait reductions by drought in %



Drought had a negative impact on all traits

- Little reduction in height, ear bearing tillers and „Marvin“ traits
- Stronger reduction in biomass components, GY stronger reduced than StrawY
- Very similar result as in spring barley

Spring barley 26 accessions					
BM	GY	HI	Hgt	StrY	TN
-22	-29	-11	-9	-17	19